THE EFFECTS OF MEPERIDINE HYDROCHLORIDE AND CODEINE PHOSPHATE ON THE GROWTH AND MOTILITY OF TETRAHYMENA PYRIFORMIS

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It is hypothesized that since the organism has acetylcholine and acetylcholinesterase activity and since these drugs are inhibitory to these activities in nervous systems of higher forms of life, such relationships may exist on the excitability of this unicellular organism. This would be a significant finding in cellular physiology.

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CHAPTER I

INTRODUCTION

Meperidine hydrochloride, a synthetic morphine-type drug, and codeine phosphate, an opium derivative morphine-type drug, are known analgesics used in the relief of pain (Huff et al., 1970). These opiate drugs and their synthetic derivatives have been known to have a direct effect upon the nervous systems of higher forms of life (Huff et al., 1970). This experimental work was concerned with the cellular action of these drugs on the growth and motility of the protozoan, Tetrahymena pyriformis.

A common entity shared by T. pyriformis and the vertebrate is that both possess acetylcholine (Ach) and acetylcholinesterase (Ach-ase) activity (Seaman, 1951). Seaman and Houlihan (1951) reported that acetylcholine was split by this organism. Also, acetylcholinesterase activity in this ciliate is reported to be localized in the fibrillar system of the pellicle.

Florey (1966) states that vertebrate transmission for motor end-plates and synapses between pre- and post ganglionic neurons is accomplished by a release of a transmitter substance called acetylcholine. He further states that the rate of removal of the transmitter is greatly speeded up by the action of a hydrolyzing enzyme, Ach-ase.

Seaman (1951) states that Ach and Ach-ase activity in the Tetrahymena is localized in the fibrillar system of the pellicle. Upon differential centrifugation, he obtained a fraction which contained

only enzymatic activity from the pellicle. He then stated that his fraction contained the entire amount of acetylcholinesterase activity of the organism.

The question raised as to whether or not the drugs mentioned affect acetylcholine and acetylcholinesterase activity has been answered for several vertebrate systems. According to Grubber et al. (1941), in 1939 Eisleb and Schaumann reported that in mice meperidine hydrochloride in a $1{:}5 \times 10^6$ dilution antagonized the effects of acetylcholine on the blood pressure; the spasm of smooth muscle; the action of histamine on the gut and bronchioles; and the effects of epinephrine on kidney vessels.

Young et al. (1955) demonstrated that codeine phosphate caused no inhibition of cholinesterase activity in bovine erythrocytes, rat brain, dog serum and the muscular wall of dog intestine. He further stated that the analgesic effectiveness of this drug probably does not depend upon the anti-cholinesterase activity.

Hypothetically, the same antagonistic effects of meperidine hydrochloride on acetylcholine as observed in the vertebrate could possibly have the same effects in Tetrahymena. Also, one could hypothesize the effect noted for codeine phosphate on acetylcholinesterase and acetylcholine as observed in the vertebrate systems, is probably similar to the effect it has in Tetrahymena.

It is clear that there are a number of questions that have to be answered in order to clearly understand the physiological effects of these drugs. It is the purpose of this research to answer some of the questions by observing physiological changes related to growth and to

observe the drugs' effects on motility. This research could provide significant information in cell physiology by helping to answer questions relating to the long-range effects drugs have on the nervous systems and motion in higher forms.

CHAPTER II

REVIEW OF LITERATURE

The physiological effects of certain prescribed drugs have been the target of many research investigations during recent years. The Protozoa have been widely used in these studies with the ciliate Tetrahymena pyriformis, an especially selected form in many of these experiments.

Two drugs were used in this investigation: meperidine hydrochloride and codeine phosphate. Meperidine hydrochloride is a synthetic morphine-like drug that is an analgesic and sedative (Huff et al., 1970). Codeine phosphate is an opium derivative morphine-like drug that is an analgesic and a sedative (Huff et al., 1970).

The reported work concerning the medical usage and physiological effects of these drugs has been limited to the vertebrates. Clark (1947) performed a comparative study of meperidine hydrochloride on the gastric secretion in the dog; Szerb (1953) observed the effects of meperidine hydrochloride and codeine on the circulating eosinophils of mice; Sinclair (1972) observed the effects of meperidine hydrochloride in rabbits; Ernst (1938) observed the minimal dosage effects of codeine and morphine in depressing the cough reflex in cats; and Huggins and Handley (1949) observed the effects of codeine on the blood flow in dogs.

These opiate drugs and their synthetic derivatives are known to have a direct effect on the nervous systems.

Grubber et al. (1941) observed that meperidine hydrochloride will antagonize the effects of acetylcholine in several animal systems. Young et al. (1955) reported that codeine phosphate caused no inhibition of cholinesterase activity from bovine erythrocytes in several vertebrate systems.

According to Manwell (1968), in 1914, Sharp reported observing a neuromotor apparatus in the ciliates. Seaman (1955) contends that the presence of acetylcholine and acetylcholinesterase activity in Tetrahymena is involved in the nerve control of this neuromotor apparatus.

Tetrahymena pyriformis was the protozoan of choice in these experiments because of the extensive research that has produced biochemical, physiological, and nutritional information. Hill (1972) states that the ciliate, Tetrahymena, in its nutrition, morphology, and reproduction is similar to a great number of organisms in this phylum, which consists of 6000 reported species.

When administering some drugs to the protozoa, several factors have been previously observed. Van Eys and Warnock (1963) observed that the methonium drugs inhibit the motility of the ciliate, Spirostomum, causing cytolysis and often resulting in complete disintegration.

Jones and Baker (1946) observed aggregation of Tetrahymena when adding the malarial drug, phenanthiaquinone. It was found that aggregation of Tetrahymena can be accomplished by adding this drug.

Jones and Jahn (1965) observed the effects of hexamethonium chloride, a ganglionic blocking agent, on the ciliary and contractile

systems of the ciliate Spirostomum. Results showed that fed organisms, as opposed to starved ones, were more susceptible to the drug action of this metabolite. On the contrary, Kidder and Stuart (1939), performing growth studies on the ciliate Colpoda, stated that the available food supply is one of the principal factors operating to limit the population of this ciliate.

Sanders and Nathan (1959) observed the effects of antihistamines on the growth and motility of Tetrahymena logarithmic growth. Results indicated that the antimotility and growth effects observed were quantitatively similar to the local anaesthetic properties of the antihistamines.

Several studies have been reported on drugs and their effects on the growth and motility of Tetrahymena. Frankel (1970) studied the recovery of Tetrahymena from the inhibitory effects of cycloheximide. He found that cycloheximide in the medium of recovering cells was able to inhibit cells that had not been previously exposed to the drug. Satir (1967) observed that actinomycin D caused a period of growth reduction, the lag phase lengthen, and the cell concentration at which the Tetrahymena entered the stationary phase was reduced. Rosenbaum and Carlson (1969) used colchicine at varied concentrations to inhibit the regeneration of cilia from deciliated Tetrahymena.

According to Hill (1972), the number of papers published on Tetrahymena pyriformis increased enormously when axenic cultures of Tetrahymena were established. Prescott (1972) states that Tetrahymena was the first protozoan that could be grown in a medium free of bacteria. The method of maintaining axenic cultures in this investigation was the

modified method of Mackinnon and Hawes (1961) using a sterilized proteose-peptone medium with the addition of streptomycin.

Meperidine hydrochloride and codeine phosphate were used to determine if the effect on the nerve function in higher vertebrates could be repeated in the cilia action of a unicellular organism.

CHAPTER III

MATERIALS AND METHODS

Axenic cultures of Tetrahymena pyriformis, Lot No. 9a, were obtained from the Carolina Biological Supply Company, Burlington, North Carolina.

The organism was cultivated in a medium recommended by Carolina Biological Supply Company for Tetrahymena. Table 1 gives a listing of the medium components.

Table 1. Composition of Tetrahymena medium

Components	Amount
Proteose	5.0 g
Tryptone	5.0 g
K_2PO_4	0.4 g
Distilled Water	1.0 liter

All cultures were kept in the dark in a covered Sero-Utility Water Bath at 28 C. Because photoperiodic effects have been demonstrated (Willie and Ehret, 1968), it was suggested that cultures should be kept in the dark. Also, light can cause the destruction of essential nutrients, such as folic acid and vitamins, thus limiting growth (Kidder and Dewey, 1951), the flasks of medium were kept in the dark.

Fifty mg/ml stock solutions of meperidine hydrochloride were

obtained from the Winthrop Laboratories. Stock bottles containing 3.54 g of codeine phosphate were obtained from the Merck Chemical Company. Codeine phosphate was diluted to give a 50 mg/ml stock solution (Huff et al., 1970).

All cultures were placed in Spectronic 20 test tubes for the turbidity determinations of cell growth using the Bausch and Lomb Standard Model Spectronic 20. The wavelength was set at 450 nanometers (Everhart and Ronkin, 1966).

Studies for the quantity of motility were made using the Sedgwick-Rafter plankton counting chamber in conjunction with a Whipple-Hauser ocular micrometer disc (Scherbaum, 1957).

Direct counts of cell motility studies were made with an Aristo Four Figure-Hand Tally.

Wild-Heerbrugg Metallurgical, Inclined Binocular Body, Model 33575 Light Microscope, using 10X eyepiece was used in motility observations.

Nutrient agar (Difco) served as a detector of aerobic contamination of bacteria in stock cultures. Thioglycolate broth medium (Difco) served as a detector of anaerobic contamination of bacteria.

Inoculations for each test were made with 1.0 ml Corning disposable pipets.

The Tetrahymena medium, with the pH adjusted to 7.2, as suggested by Prescott (1958), was dispensed in 5.0 ml amounts in foam-plugged culture tubes and autoclaved at 15 lbs/in^2 at 120 C for 10 min. This reduced autoclaving time was recommended by Prescott (1972) to prevent charring of the medium and the breakdown of complex molecules. The

culture tubes were then inoculated with 1.0 ml of axenic organisms in Tetrahymena medium. These culture tubes served as the source from which organisms were taken for experimental testing. The medium was dispensed in 250 ml volumes in Erlenmeyer flasks and autoclaved under the same conditions. The flasks were inoculated with 25 ml of organisms. These flasks served as stock cultures.

Optimum bacteriological sterile conditions were maintained during each test. Cultures were axenically maintained by a modified method of Mackinnon and Hawes (1961). For stock cultures, 250 ml of sterile medium was inoculated with 25 ml of the organisms in Tetrahymena medium and 1.0 g streptomycin sulfate added. The mixture was kept in the dark in a water bath for 24 hr.

The test for bacterial contamination was made by inoculating nutrient agar plates with 0.5 ml of the organisms (Prescott, 1972). In the case of bacterial contamination, the initial axenic maintenance procedure was repeated with one modification; the 25 ml of the organisms in medium was taken from the flask containing the initial streptomycin medium and organisms. After this second inoculation, contamination was usually absent. Periodic tests, every four days, were made to insure that the axenic cultures were uncontaminated.

Stock drug solutions of meperidine hydrochloride and codeine phosphate were diluted to several arbitrary concentrations. The dilute solutions were stored at room temperature in screw-cap volumetric flasks (Stecher et al., 1968). The concentrations of the drugs used in this investigation are present in Table 2.

Table 2. Drug concentrations used in the growth and motility studies.

Meperidine hydrochloride mg/ml	Codeine phosphate mg/ml
0.25	2.75
0.45	4.50
0.65	6.50
0.85	8.50
0.90	9.50
1.50	10.00
3.00	15.00
4.50	20.00
5.50	25.00
6.50	30.00
7.50	40.00

A. Growth studies technique

Growth studies were conducted according to the modified method of Rosenbaum and Carlson (1969). All organisms used in the tests were transferred during the logarithmic growth phase (Prescott, 1957). This logarithmic growth phase was determined to be 2 to 3 days after stock culture inoculations.

Turbidity readings for both the normal growth and drug effects upon growth were measured spectrophotometrically. Optical density readings were initially taken at 4 hr intervals but the generation time

indicated by the optical density readings was not definable by this method. Optical density readings were then taken 12 hr after inoculation and subsequent readings were taken every 24 hr.

To observe the effects of the drug on growth, 1.0 ml of the organisms in Tetrahymena medium, and 1.0 ml of the drug at the given concentrations were transferred to 3.0 ml of the Tetrahymena medium.

B. Motility studies technique

Motility observations were performed according to the modified method of Scherbaum (1957). All organisms used in the test were taken during the logarithmic growth phase. To observe the effects of the drugs on motility, 0.5 ml of the stock culture of the organisms in medium and 0.5 ml of the drug solution at the varied concentrations (see Table 2) were placed in the chamber and stirred. The Tetrahymena were observed using a Sedgwick-Rafter plankton counting chamber in conjunction with a Whipple-Hauser ocular micrometer disc and light microscopy. Percent immobilization counts were made at 5 min intervals for a period of 50 min. Percent immobilization determinations were determined using the following formula:

$$\%_I = \frac{\text{Total No. of } T_I}{\text{Total No. of } T} \times 100$$

$\%_I$ = Percent immobilized Tetrahymena pyriformis

T_I = Immobilized Tetrahymena

T = Tetrahymena in chamber

The direct cell counts were made with a tally counter. Normal motility was observed by placing 1.0 ml of the organism in medium in the counting chamber. This served as the control. The chamber

containing the organisms and the drug were kept for 24 hr at room temperature in the dark. The percent immobilizations were then recorded.

CHAPTER IV

EXPERIMENTAL RESULTS

The Effects of Meperidine Hydrochloride Upon the Growth of Tetrahymena pyriformis

This test was designed to observe the effects of varied concentrations of meperidine hydrochloride on the growth of the organism. Each concentration, indicated by optical density readings, had a reduction in growth proportional to time. The drug concentrations of 0.25 mg/ml and 0.45 mg/ml reduced the growth rate approximately by one-half. Drug concentrations of 0.75-1.50 mg/ml reduced the growth rate approximately three-fourths time. Drug concentrations of 5.50-7.50 mg/ml at 24 hr became lethal to the organism. The results are given in Table 3. Concentrations of the drug are plotted against time to show this relationship to growth in Figure 1.

The Effects of Codeine Phosphate Upon the Growth of Tetrahymena pyriformis

This test was designed to observe the effects of varied concentrations of codeine phosphate on the growth of the organism. Results showed that during the time interval, 12-48 hr, the growth rate decreased in the drug-treated organism when compared with the controls. During the 48 hr interval the drug concentrations from 10-40 mg/ml induced an increase in the growth rate that surpassed the control. The results are given in Table 4. This relationship is illustrated graphically in Figure 2.

Table 3. The effects of meperidine hydrochloride upon the growth of Tetrahymena pyriformis.

Drug Concentration (mg/ml)	Optical density of growth cultures Time (hr)					
	12	24	48	72	96	120
Control	0.042	0.058	0.089	0.124	0.132	0.135
0.25	0.029	0.034	0.044	0.065	0.077	0.052
0.45	0.025	0.028	0.040	0.048	0.065	0.056
0.75	0.021	0.024	0.024	0.038	0.044	0.045
0.90	0.018	0.020	0.024	0.033	0.025	0.031
1.50	0.018	0.019	0.035	0.029	0.038	0.037
3.00	0.020	0.022	0.035	0.017	0.013	0.014
4.50	0.019	0.017	0.020	0.016	0.010	0.022
5.50	0.008	0.002	0.0	0.0	0.0	0.0
6.50	0.005	0.007	0.0	0.0	0.0	0.0
7.50	0.007	0.0	0.0	0.0	0.0	0.0

Fig. 1. Graph indicating the effects of various concentrations of meperidine hydrochloride (concentrations ranging from 0.25-7.50 mg/ml) on the growth of Tetrahymena pyriformis. Each point represents the optical density of an average of two to four experiments.

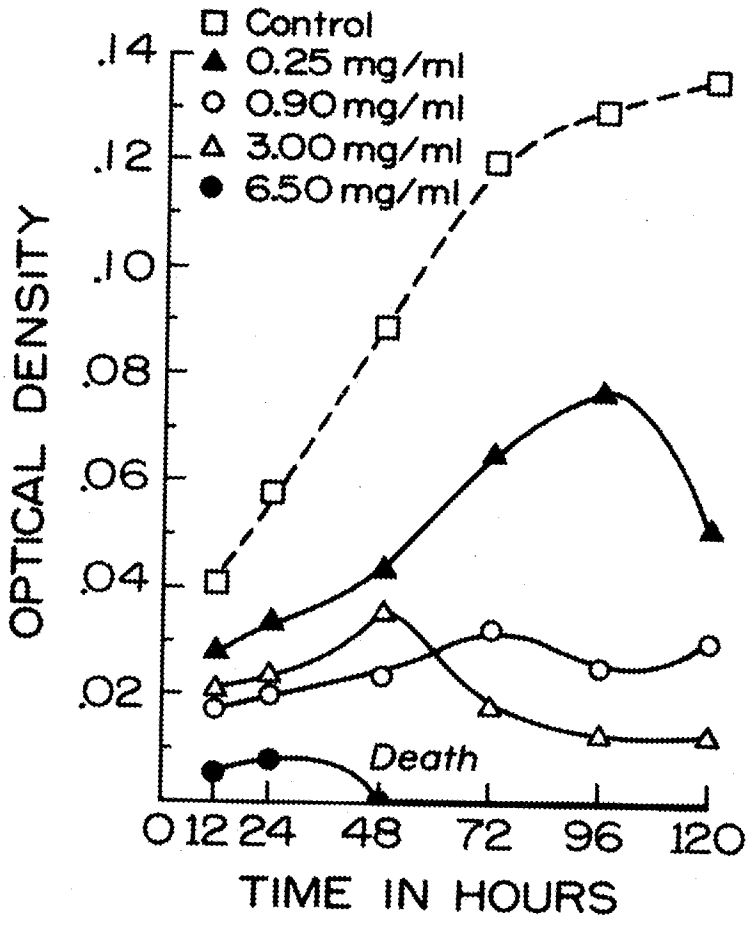
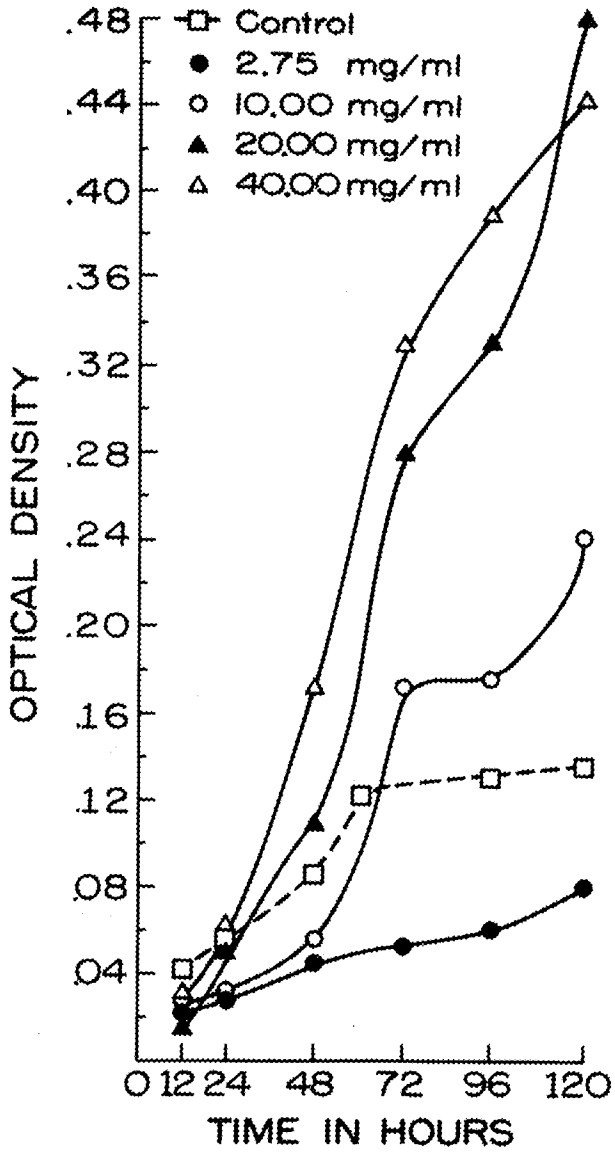

Control
0.25 mg/ml
0.90 mg/ml
3.00 mg/ml
6.50 mg/ml
.14
.12
.10
.08
.06
.04
.02
OPTICAL DENSITY
Death
0 12 24 48 72 96 120
TIME IN HOURS

Table 4. The effects of codeine phosphate on the growth of Tetrahymena pyriformis.

Drug Concentration (mg/ml)	Optical density of growth cultures Time (hr)					
	12	24	48	72	96	120
Control	0.042	0.058	0.089	0.124	0.132	0.135
2.75	0.023	0.029	0.045	0.053	0.060	0.081
4.50	0.035	0.025	0.030	0.040	0.050	0.077
6.50	0.025	0.025	0.025	0.041	0.049	0.064
8.50	0.023	0.23	0.30	0.055	0.068	0.074
9.50	0.030	0.031	0.026	0.065	0.089	0.088
10.00	0.027	0.032	0.059	0.173	0.178	0.244
15.00	0.024	0.023	0.098	0.253	0.328	0.463
20.00	0.019	0.051	0.110	0.283	0.330	0.483
25.00	0.018	0.043	0.103	0.348	0.350	0.395
30.00	0.013	0.018	0.143	0.347	0.375	0.445
40.00	0.028	0.063	0.173	0.333	0.393	0.448

Fig. 2. Graph indicating the effects of various concentrations of codeine phosphate (concentrations ranging from 2.75-40.00 mg/ml) on the growth of Tetrahymena pyriformis. Each point represents the optical density of an average of two to four sets of experiments.


.48
.44
.40
.36
.32
.28
.24
.20
.16
.12
.08
.04
OPTICAL DENSITY
Control
2.75 mg/ml
10.00 mg/ml
20.00 mg/ml
40.00 mg/ml
0 12 24 48 72 96 120
TIME IN HOURS

The Effects of Meperidine Hydrochloride on the Motility of Tetrahymena pyriformis

Concentrations of 0.25-0.90 mg/ml had no effect until 24 hr after incubation. The degree of immobilization increased proportionally with the drug concentrations from 1.50-7.50 mg/ml, and the lethal dose of the drug, after 30 min, was determined to be 7.50 mg/ml (Table 5). After 24 hr all concentrations of the drug caused an increase in immobilization proportional to the concentrations (Table 6).

There was always an overlap in the reaction below the level where 100% immobilization occurred; that is, cytolysis started in some animals while others were still motile. Aggregation was also constantly observed. The organisms in the control chamber maintained a distinctive motility pattern. The drug-treated motile animals had no such pattern and the drug-treated immobilized animals were extremely fragile and any agitation of the culture caused disintegration.

The Effects of Codeine Phosphate on the Motility of Tetrahymena pyriformis

Direct cell counts indicated that the organism was not immobilized at varying concentrations of the drug until 24 hr of incubation. The results are given in Table 7.

The organisms after 24 hr were extremely fragile and any agitation of the culture caused disintegration. After 24 hr cytolysis had started in some animals while others were still motile. Aggregation occurred after 24 hr. A lethal dose of the drug was not established.

Table 5. The effects of meperidine hydrochloride on the motility of Tetrahymena pyriformis.

Drug conc.	Per cent immobilization occurring with time (min)									
(mg/ml)	5	10	15	20	25	30	35	40	45	50
0.25	0	0	0	0	0	0	0	0	0	0
0.45	0	00	0	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0	0	0	0
0.90	0	0	0	0	0	0	0	0	0	0
1.50	0	0	0	0	0	5	7	12	16	17
3.00	0	0	0	0	23	29	38	51	49	53
4.50	0	0	12	25	36	42	55	67	70	78
5.50	0	16	32	48	57	80	88	86	95	98
6.50	0	22	50	66	70	92	95	97	96	98
7.50	0	31	51	74	97	100	100	100	100	100
Control*	0	0	0	0	0	0	0	0	0	0

*Culture medium without drug

Table 6. The effects of meperidine hydrochloride on the motility of Tetrahymena pyriformis.

Drug concentrations (mg/ml)	Immobilization after 24 hr % Immobilization
0.25	55
0.45	57
0.75	60
0.90	75
1.50	83
3.00	96
4.50	100
5.50	100
6.50	100
7.50	100
Control*	10

*Culture medium without drug

Table 7. The effects of codeine phosphate on the motility of Tetrahymena pyriformis.

Drug concentrations (mg/ml)	Immobilization after 24 hr % Immobilization
2.75	41
4.50	42
6.50	43
8.50	43
9.50	46
10.00	63
15.00	83
20.00	88
25.00	91
30.00	94
40.00	90
Control*	10

*Culture medium without drug

CHAPTER V

DISCUSSION AND CONCLUSION

Several factors must be considered when one observes the effects of drugs or any type of inhibitor on Tetrahymena pyriformis. First, is the question of penetration. For a number of drugs the concentrations required to achieve a given penetration effect are much higher for Tetrahymena than for mammalian cells. The ciliate cortex is a complex surface structure composed in many places of three unit membrane layers in close approximation to one another. This may represent a substantial barrier to the entry of molecules from the outside. There has been and still remains the controversy as to whether uptake of nutrients and other materials occurs across the surface membrane or only by means of the oral apparatus (Prescott, 1972).

Although turbidity determinations were used in determining the growth rate, it is well known that the age and number of cells present in the culture can influence the cell count at any specified time. Nonetheless, the turbidity determination is the most satisfactory method to ascertain density based on growth according to Gross (1955). The rate of growth during the exponential increase remains unaffected by the size of the inoculum and is considered to be more constant and reproducible than other growth indices.

When one observes the effects of drugs on an organism, the chemistry of the drug is an added parameter. Meperidine hydrochloride has an atomic weight of 283.79 g, whereas codeine phosphate has an

atomic weight of 424.38 g. The molecular size differences may suggest differences in penetration rates. Therefore, more inhibitory effects of meperidine hydrochloride on growth and motility would be predicted. It would seem that meperidine hydrochloride caused permeability of the body wall. Nathan and Friedman (1962) showed that chlorpromazine, a tranquilizer, caused immobilization of Tetrahymena pyriformis and increased the permeability of the body wall.

On the contrary, it can be postulated that codeine phosphate stimulated growth and had no effect on motility. One could hypothesize that codeine phosphate possesses a growth factor that can be used by the Tetrahymena.

The inhibitory growth effects of meperidine hydrochloride and the stimulating effects of codeine phosphate may have a direct neurological action in Tetrahymena.

Seaman (1955) states that acetylcholine and acetylcholinesterase is present in certain ciliates. This evidence supports a "neuroid" analogy for the "kinetoderma" of ciliates. Seaman suggests that the same direct relationship exists between ciliary activity and acetylcholinesterase as there is between acetylcholinesterase activity and nerve control. He cites in this connection that acetylcholinesterase and acetylcholine have been found in Tetrahymena geleii. Additionally, Manwell (1968) reports that in 1914 Sharp observed a neuromotor apparatus in the ciliates.

In all cases, motility was inhibited by meperidine hydrochloride at varying times and motility was inhibited by codeine phosphate at 24 hr. Since Seaman (1951) reported that acetylcholine was involved in

impulse transmission along the rows of cilia, an inhibitor of acetylcholine such as that of meperidine hydrochloride and codeine phosphate could cause immobilization.

According to Grubber et al. (1941), in 1939, Eisleb and Schaumann reported that meperidine hydrochloride could antagonize the effects of acetylcholine on several systems in mice. Transferring this finding to the Tetrahymena, an inhibitor of acetylcholine such as that of meperidine hydrochloride would have an effect on the motility and growth rates.

Young et al. (1955) demonstrated that codeine phosphate caused no inhibition of cholinesterase activity on bovine erythrocytes. He further stated that the analgesic effectiveness of this drug probably does not depend on its anti-cholinesterase activity, since the more potent analgesics do have inhibitory effects on cholinesterase activity. Since codeine phosphate did not cause immobilization in Tetrahymena until after 24 hr, one could suggest that this drug did not inhibit the acetylcholine activity of the organism. The immobilization occurring after 24 hr might very well be due to food depletion in the medium. Kidder and Stuart (1939), performing growth studies on the ciliate Colpoda, stated that the available food supply limits the population of this ciliate. On the other hand, Jones and Jahn (1965) observed Spirostomum ciliary movement and found that fed animals were susceptible to the drug action of hexamethonium chloride than the starved ciliates.

Immobilization was a forerunner of cytolysis and disintegration. Van Eys and Warnock (1963) observed similar results with Tetrahymena.

They found that the methonium drugs, which inhibited the motility of the ciliate, caused cytolysis and subsequent disintegration. They suggested that a possible cause could be the binding of hexamethonium chloride at the cortical surface, causing displacement of ions, and disruption of the membrane.

The organisms in the control chamber showed a distinct pattern formation whereby the organisms seem to form patterns similar to geometric figures. Jahn and Brown (1961) observed that in a dense culture and in a shallow medium depth, Tetrahymena pyriformis moved in streams that formed patterns. The streams were horizontal and formed a polygonal network, with four or five streams meeting at each apex of the net.

Aggregation of the organism occurred after adding the drugs during the motility experiments. When meperidine hydrochloride was added, aggregation occurred after 20 min for concentrations ranging from 0.90-7.50 mg/ml. On the other hand, aggregation was absent when codeine phosphate was added. Jones and Baker (1946) observed aggregation of the Tetrahymena when the malaria drug phenanthiaquinone was added to the culture.

From the experimental results it is seen that meperidine hydrochloride inhibited acetylcholine activity which in turn caused a reduction in growth and immobilization in Tetrahymena pyriformis. Codeine phosphate did not inhibit acetylcholine activity but stimulated growth and additionally had no effect on motility until after 24 hr exposure to the drug.

Immobilization occurring after 24 hr may have been due to food

depletion (Kidder and Stuart, 1939). The cytolysis and disintegration that occurred could be due to the drugs inhibitory property acting on the cellular cortex (Van Eys and Warnock, 1963).

Aggregation was constantly observed during motility observations. Aggregation of the organisms could have resulted from the drug stimulation produced in the system (Prescott, 1972).

CHAPTER VI

SUMMARY

1. Meperidine hydrochloride at all concentrations reduced the growth rate of Tetrahymena pyriformis. Meperidine hydrochloride at concentrations of 5.50-7.50 mg/ml are lethal to Tetrahymena pyriformis.
2. Codeine phosphate at concentrations of 10-40 mg/ml caused an increase in the growth rate of Tetrahymena pyriformis.
3. Twenty-four hr were required for meperidine hydrochloride at concentrations of 0.25-0.90 mg/ml to immobilize Tetrahymena pyriformis. The drug concentrations of 1.50-7.50 mg/ml caused immobilization during the 50 min observations.
4. Twenty-four hr were required for codeine phosphate at concentrations of 2.75-40.00 mg/ml to immobilize Tetrahymena pyriformis.

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